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THE INHIBITION OF THE LOCAL SHWARTZMAN REACTION BY HEPARIN

LEIGHTON E. CLUFF* AND MORGAN BERTHRONG

Departments of Medicine and Pathology, The Johns Hopkins University School of Medicine and the Hospital of the Rockefeller Institute for Medical Research

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Since the observations by Shwartzman (1) of the remarkable phenomenon now bearing his name, great efforts have been directed toward the elucidation of its pathogenesis. This peculiar focal skin necrosis which occurs in rabbits as well as other animal species upon the introduction of bacterial endotoxins has remained inadequately explained. It is recognized that the reaction depends upon the proper preparation of the skin site by the intradermal injection of small amounts of bacterial endotoxin. Twenty-four hours after such an injection, the same bacterial endotoxin is injected intravenously in somewhat larger amounts and within 2 to 6 hours a hemorrhagic necrosis will develop at the prepared skin site in a large percentage of the rabbits used. While only a limited number of agents, largely bacterial endotoxins, may be used to prepare the skin, a wider variety of materials may be injected intravenously to provoke the local reaction once proper preparation has been effected. Successful preparation of the skin is attended by a local inflammatory reaction, yet the Shwartzman reaction which follows the provocative intravenous injection is not proportional to the degree of that inflammation. Other substances which are capable of producing profound local inflammatory changes do not prepare the skin for this reaction. Within susceptible species, individual animals which are resistant to the reaction are relatively common. Resistance to the reaction may be also acquired and this is thought to depend upon alterations in the reticulo-endothelial system (2, 3). The phenomenon appears to be unrelated to the usual concepts of the antibody-antigen reaction.

The characteristic features of the local Shwartzman reaction are extensive hemorrhages, exudation of leucocytes, edema and venous and capillary thromboses at the skin site. These develop within about two hours of the provocative injection and are followed by local necrosis of both the epidermis and dermis with eventual sloughing. While thromboses have been prominently described by all workers since the earliest morphological observations, greater emphasis has usually been placed upon the hemorrhagic manifestations. It is thought that following the provocative injection vascular damage occurs in the area of previous preparation, and hemorrhage with leucocytic exudation ensues.

* Present Address: Rockefeller Institute for Medical Research, New York. Aided by a Fellowship from the National Foundation for Infantile Paralysis.

Thrombi are thought to form on the damaged vascular endothelium. Recently Stetson (4) has suggested that thromboses are of primary importance. Our own histopathological study of the local Shwartzman reaction has also impressed us with the possible fundamental importance of thrombus formation in the development of the reaction and led us to seek the effect of anticoagulants on the development of this phenomenon.

MATERIALS AND METHODS

Two to three kilogram albino and hybrid rabbits have been used. The toxins employed were the P-25 polysaccharide of *Serratia marcescens*[1] and meningococcus culture filtrate[2]. Fifty to one-hundred micrograms of the P-25 polysaccharide were injected intradermally to prepare the skin and 125 to 200 micrograms were used as the provocative intravenous doses 24 hours later. A 1:2 dilution of the meningococcus culture filtrate in a 0.25 ml. intradermal injection was used for the skin preparation and 2.0 ml. of a 1:10 dilution followed as the intravenous provocative dose. In preliminary experiments these doses of toxins were found to produce reactions in about 60% of tested animals. In most experiments, control animals injected simultaneously with the heparinized animals showed the expected rate of positive reactions. The heparinized animals could be considered, on a basis of previously tested reactivity, their own control as well. The skin of the abdomen or flanks of the rabbits was used for the tests following mechanical shaving. All intravenous injections were given via the ear veins, and ecchymoses, especially in heparin treated animals, were reduced by use of 24 or 25 gauge needles.

White blood cell counts were done using 5% acetic acid colored with gentian violet as diluent. Differential counts were made on smeared slides stained with Wright's method. Platelet counts, when done, were made using Rees-Ecker diluting fluid. All cell counts were performed in the usual manner in a bright-line hemocytometer chamber. The inaccuracy of platelet counting by this method prevented us from investigating this aspect in detail during the present study. Only gross variations in platelet counts were considered significant and even these were viewed skeptically. In many instances biopsy material of the skin reaction site was taken, fixed in 10% formalin or in Helly's solution. Sections were stained with hematoxylin and eosin and by Mallory's phosphotungstic acid-hematoxylin method.

The clotting times were determined by a modified (two-tube) Lee-White method. Blood for clotting times was drawn from ear veins just prior to making the heparin injection. The heparin solutions were aqueous preparations obtained commercially (Lederle, Ortho, and Organon) and all contained approximately 10 mgm. per ml. of the sodium salt of heparin. Heparin was injected intravenously, first in doses varying from one to five mgms. depending upon the existing clotting time, at intervals of 30 to 45 minutes. Later during the progress of the experiments, an injection of five mgms. every 30 to 40 minutes was given regardless of the extremely prolonged clotting times. The first injection of heparin was always given 5 to 15 minutes prior to the intravenous provocative dose of bacterial toxin. Heparin was always given for at least four hours after the provocative dose of toxin and occasionally for as long as twelve hours.

A positive Shwartzman reaction was considered only when gross hemorrhage occurred at the prepared skin site and when this hemorrhage was followed by gross necrosis or slough-

[1] Obtained through the kindness of Dr. M. J. Shear, National Institutes of Health, Bethesda, Maryland.

[2] Obtained through the kindness of Dr. G. Shwartzman, Mt. Sinai Hospital, New York.

ing. The hemorrhage was usually apparent at two hours and was always well developed four hours after the intravenous toxin injection. The necrosis of the skin was apparent at 24 hours while sloughing usually occurred by 96 hours.

EXPERIMENTAL RESULTS

In initial experiments, heparin was given with the purpose of causing a continued elevation of the animal's clotting times to forty minutes. This figure was arbitrarily selected as a level at which vascular thrombosis probably would not occur. The effect obtained by such heparin administration upon the development of the Shwartzman reaction in 11 albino rabbits is seen in Table I. All of these animals were known reactors, having demonstrated positive reactions before the heparin experiment. They had not been treated in such a manner as to have possibly acquired resistance. Two of the 11 animals were used for heparinization twice but were checked for continued responsiveness to the reaction between experiments. Many other rabbits were studied in a similar fashion but because of their failure to respond consistently with positive reactions in the absence of heparin treatment, were not included in the report. As can be seen in Table I, three animals that previously responded with gross hemorrhages at the skin site, failed to show hemorrhages or necroses under heparin treatment. Three others showed initially either prominent or slight hemorrhage in the prepared skin site but these hemorrhages disappeared within 24 or 36 hours without the development of the usual gross necrosis. While these observations, especially the resolution of prominent hemorrhages without necrosis formation, were of great interest and suggested a heparin effect, the numbers were too small to be of significance.

In these experiments, the clotting time determinations indicated that great variations occurred in individual responses to the anticoagulant effect of heparin, and that changes from extreme prolongation of clotting time to levels close to the normal ranges might occur with remarkable rapidity. It seemed impossible to maintain a constantly elevated clotting time in rabbits by this technique. It was also realized that in the presence of local tissue damage occurring in the prepared skin sites, an essentially incoagulable blood might be necessary to prevent local thrombosis. For these reasons, it was decided to employ higher doses of heparin and to use the clotting times only to demonstrate the effectiveness of the heparin rather than as a criterion for further doses. Animals given five milligrams of heparin prior to the intravenous toxin injection and every thirty or forty minutes for at least four hours thereafter showed on clotting time determinations, made just before subsequent heparin injections, clotting times usually greater than three hours and often the blood was incoagulable. No apparent toxic effects in control animals maintained on these dosages were noted, nor did spontaneous hemorrhages occur in control or tested animals. No significant alterations in leucocyte or platelet counts

or morphology of these cells were observed in control animals subjected to this dosage schedule.

TABLE I

Effect of Inadequate Heparinization on the Local Shwartzman Reaction

EXP. NO. AND DATE	HEPARIN TREATED (See text)					UNTREATED			
	Rabbit No.	Endotoxin	Range of Clotting Time in Minutes	Reaction		Rabbit No.	Endotoxin	Reaction	
				6 hrs. Hem.	24 hrs. Nec.			6 hrs. Hem.	24 hrs. Nec.
I, 3/30						245	P-25	+	+
						246	P-25	+	+
						247	P-25	0	0
						248	P-25	0	0
						249	P-25	+	+
						250	P-25	0	0
						251	P-25	+	+
						252	P-25	+	+
						253	P-25	0	0
						254	P-25	+	+
						255	P-25	+	+
						256	P-25	0	0
						257	P-25	+	+
						258	P-25	+	Died
						259	P-25	+	+
						260	P-25	Died	–
						261	P-25	+	+
						262	P-25	0	0
						263	P-25	0	0
II, 4/9	245	P-25	25–60	0	0	246	P-25	+	+
	249	P-25	20–90	0	0	251	P-25	+	+
	252	P-25	5–90	+	+				
III, 4/11	255	P-25	30–110	+	+	254	P-25	+	+
	259	P-25	18–120	+	+	257	P-25	+	+
	261	P-25	45–75	+	+				
IV, 4/25	245	P-25	80–180	0	0				
	246	P-25	80–180	+	0				
	251	P-25	55–120	±	0				
	252	P-25	75	+	+				
	254	P-25	60–110	±	0				
Totals	11	—	—	8 (72%)	5 (45%)	23	—	15 (65%)	14 (61%)

Thirty-one tests of this type, using 28 rabbits, were employed as well as 53 control procedures on 46 rabbits. The control experiments were performed in the same manner using the same endotoxin solutions as those testing the effect of heparin except heparin was not administered to such controls. On 12 oc-

casions the heparinized animal had exhibited, either prior or subsequent to the heparin experiment, positive Shwartzman reactivity. This testing, however, was never carried out in such a manner as to alter the subsequent reactivity of the animals for in order to produce resistance daily injections for 6 or 7 days is necessary. It is well recognized that animals showing initial Shwartzman reactivity will usually continue to give positive reactions on at least several repeated tests as long as the frequency of testing does not approach that which is known to result in resistance. When the test animals were given heparin in these high dosages, hemorrhages in significant amounts did not occur at the prepared skin sites and necrosis never ensued. (Table II). Twenty-four hours after preparation for the Shwartzman reaction, a slightly raised blanched area one to two centimeters in diameter is often seen at the cutaneous site. In control animals showing such a preparatory reaction, a prominent hemorrhagic necrosis almost always followed the intravenous injection of toxin. When such an animal was heparinized, tiny petechiae might appear in the blanched area after the provocative injection only to disappear after 12 to 24 hours without the occurrence of significant hemorrhage or the development of any necrosis. While more frequent use of the same animal for both heparin testing and control reactions would have been desirable, the frequency of positive reactions among the entire control group as compared to the absence of a single positive reaction among the heparin tested animals leaves no doubt that heparin has inhibited the development of the Shwartzman reaction.

White blood cells were counted in some of the tested animals of both the heparin treated and control groups. Counts were made prior to the injection of heparin and toxin, then again at one hour and three to four hours after the provocative injection of toxin (Figures A and B). It is suggestive that in the heparin treated animals there was less leucopenia, although differential counts were quite similar. All animals showed neutropenia at one hour. Thrombocytopenia appeared less in heparin treated animals, but, in view of the inaccuracies of the technical procedure used for counting the platelets, has not been considered significant.

Ten rabbits were selected for study of the influence of heparin on pyrogenic properties of the toxin. Eight of the rabbits were given 200 micrograms of P-25 polysaccharide intravenously. Four of these had received a prior injection of five mgm. of heparin and were maintained on the high dosage schedule of heparin used to inhibit the skin reactivity. The remaining two animals received heparin in the same dosages without toxin injections. Rectal temperatures were taken prior to all injections and every 30 to 45 minutes thereafter for four hours. During the following 20 hours, temperatures were taken at less frequent intervals. It was apparent in this small series, that no alterations in

the pyrogenicity of the toxin occurred under the influence of the high dosages of heparin. The plotted temperatures from the two groups of toxin challenged

TABLE II

Massive Heparinization and Its Effect on the Local Shwartzman Reaction

EXP. NO. AND DATE	HEPARIN TREATED (5 mgm. q. 30 min. for 4 hours.)				UNTREATED			
	Rabbit No.	Endotoxin	Reaction		Rabbit No.	Endotoxin	Reaction	
			6 hrs. hem.	24 hrs. nec.			6 hrs. hem.	24 hrs. nec.
I, 6/7	757	P-25	0	0	765	P-25	+	Died
	758	P-25	0	0	766	P-25	0	0
	759	P-25	0	0	767	P-25	0	0
	760	P-25	0	0	769	P-25	+	0
	761	P-25	Died	–	770	P-25	+	+
	762	P-25	0	0	771	P-25	+	+ Died
	763	P-25	0	0	772	P-25	+	+
	764	P-25	Died	–	773	P-25	0	0
	768	P-25	0	0	774	P-25	Died	–
II, 6/17	769	P-25	0	0	757	P-25	0	0
	770	P-25	0	0	758	P-25	0	0
	772	P-25	petechiae	0	759	P-25	+	0
					760	P-25	0	0
					762	P-25	0	0
					763	P-25	+	+
					768	P-25	+	+
III, 6/23	759	P-25	0	0	769	P-25	+	0
	763	P-25	0	0	770	P-25	0	0
	768	P-25	0	0	772	P-25	+	+
IV, 8/19					1	P-25	0	0
					2	P-25	+	+
					3	P-25	+	+
V, 8/22	2	P-25	petechiae	0				
	3	P-25	petechiae	0				
	4	P-25	petechiae	0				
VI, 8/25					1	P-25	0	0
					4	P-25	0	0
					5	P-25	+	+
					6	P-25	+	+
					7	P-25	+	+
					8	P-25	+	+
VII, 9/5	5	P-25	0	biopsied				
	9	P-25	0	biopsied				
	10	P-25	0	biopsied				
	18	P-25	0	biopsied				
	19	P-25	0	biopsied				

TABLE II—*Continued*

EXP. NO. AND DATE	HEPARIN TREATED (5 mgm. q. 30 min. for 4 hours.)				UNTREATED			
	Rabbit No.	Endotoxin	Reaction		Rabbit No.	Endotoxin	Reaction	
			6 hrs. hem.	24 hrs. nec.			6 hrs. hem.	24 hrs. nec.
VIII, 9/9					5	P-25	+	+
					9	P-25	+	+
					10	P-25	0	0
					18	P-25	+	+
					19	P-25	+	+
IX, 7/13	a	P-25	0	0	f	P-25	+	+ Died
	b	P-25	0	0	g	P-25	+	+
	c	P-25	0	0	h	P-25	+	+
X, 8/5					d	P-25	+	+
					e	P-25	+	+
					i	P-25	+	+
					j	P-25	+	+
					v	P-25	+	+ Died
					w	P-25	+	+ Died
					x	P-25	0	0
					y	P-25	0	0
XI, 8/7	d	P-25	petechiae	0	i	P-25	+	+
	e	P-25	petechiae	0	j	P-25	+	+
XII, 7/17					r	mening.	+	+
					s	mening.	+	+
					t	mening.	+	+ Died
					u	mening.	+	+ Died
XIII, 7/22	l	mening.	petechiae	0 Died	o	mening.	+	+
	m	mening.	petechiae	0 Died	p	mening.	+	+ Died
	n	mening.	0	0	q	mening.	+	+
Totals....	31	—	0 (8 petechiae)	0	53	—	38 (71%)	24 (62%)

animals yielded superimposable curves. There was no effect upon the normal rabbit temperature in the two animals given heparin alone.

No special studies have been made to ascertain the influence of heparin on the lethality of the toxin. From observations made on animals thus far studied, however, no difference was apparent in the mortality rates among animals treated with heparin and those used as controls. After injection of toxin, the heparinized animals showed as much dyspnea, diarrhea, cyanosis, and conjunctival hyperemia as did the controls, in spite of the complete inhibition of the local skin reaction. No systemic hemorrhagic manifestations were noted

in those heparinized animals dying following toxin administration. Ecchymoses at the site of venepunctures were the only hemorrhagic manifestations noted.

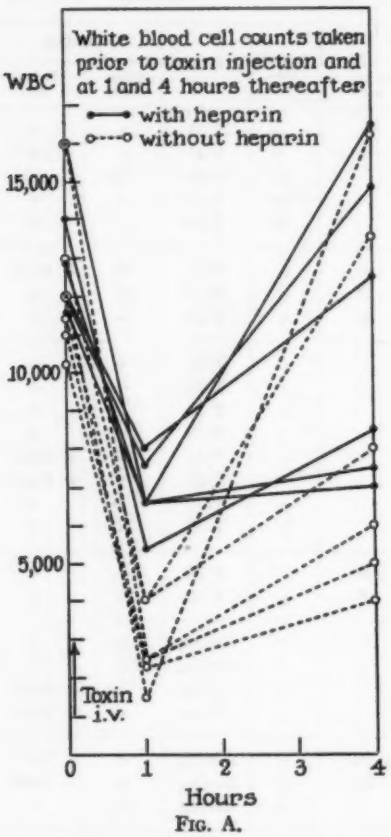

FIG. A.

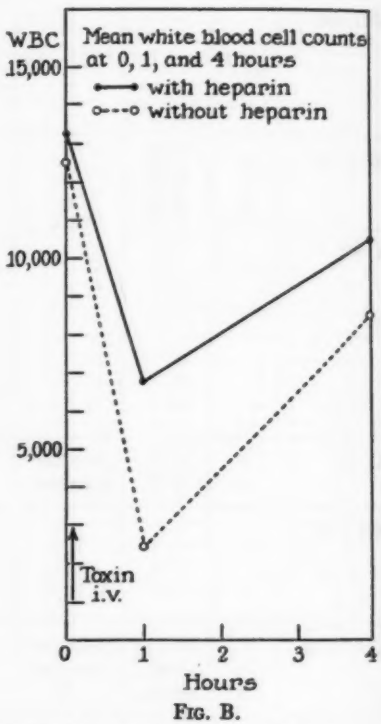

FIG. B.

Response of circulating leucocytes to intravenous injection of endotoxin, with and without heparinization.

DISCUSSION

While it has long been considered that the local Shwartzman reaction is closely related to a peculiar damage to blood vessel walls produced by the skin preparation, the greatest importance has been attached to the hemorrhages occurring through the damaged vessels following the provocative intravenous dose. Indeed heparin was used, although without success, to induce the reaction in animals prepared with potent bacterial endotoxins (1). It was probably thought that in the presence of the vascular damage, heparin alone might initiate the reaction. Recently Stetson has considered the development of the

thromboses of primary importance (4). The complete inhibition of virtually all manifestations of the local Shwartzman phenomenon by heparin is of distinct interest in this regard. At a time when the blood has been made incoagulable by the administration of heparin no significant hemorrhages occurred at the prepared skin sites following the injection of intravenous toxin. In the absence of experiments with other anticoagulants, which are to be undertaken, it is impossible to say that this inhibitory influence is due solely to the anticoagulant activity of heparin. Indeed, in dealing with a substance such as heparin, which has multiple effects upon biological reactions, great caution must be used in assuming that its effect upon a specific phenomenon is due to one of these many functions. Heparin is known to neutralize serum complement. It alters the isoagglutinin titer of serum. It prevents the action of certain enzyme systems. In high concentrations, probably higher than would be obtained by our doses in rabbits, it also acts on plasma proteins and influences the sedimentation rate of red cells. Much further work will be necessary to elucidate the fundamental mechanism behind the observations reported here.

It may be important to point out, however, that heparin in doses large enough to alter significantly the coagulation time but not administered in such a way as to maintain constantly elevated clotting times at very high levels was not effective in abolishing the reaction. Thus, relatively large doses of heparin, totaling as much as 19 mgm. given over a four hour period, failed to alter the local reaction in rabbits. It must also be noted that heparin in doses sufficient to inhibit the local reaction failed to alter significantly the pyrogenicity of the toxin and failed to inhibit the prominent systemic effects of the provocative intravenous dose such as the diarrhea, dyspnea, and prostration. Studies of the possible heparin effect upon the lethality of the toxin have not been made, but several animals died during the course of our experiments following the routine intravenous provocative dose in spite of heparin treatment. Heparin modified slightly but did not inhibit the leucopenic effect of the toxin. These latter observations seem to indicate that the inhibitory effect of heparin could not be the result of a direct inactivation of the toxin *in vivo*, nor has it protected the animal from the systemic effects of the intravenous toxin.

Histological studies of the prepared skin sites from those animals in which the Shwartzman reaction had been inhibited by heparin showed, at 6 hours after the intravenous provocative dose, a remarkable absence of the thromboses, edema, and hemorrhages seen to be so severe in the positive controls at the same time (Plates I and II). A very rare tiny dermal hemorrhage was seen in an occasional animal protected by heparin, comparable to that seen grossly. Early necrosis of tissue cells and leucocytes was also absent in the heparin treated animals in which the sections of the skin sites taken 6 hours after the intravenous injection of endotoxin were identical to skin sites biopsied 30 hours after similar preparation with P-25 toxin but before any intravenous

toxin had been administered (Plates I and III). No differences in the degree of leucocytic infiltration of the tissues was observed in the heparin treated animals save for an absence of the prominent perivascular exudate seen in the positive reactions of the controls. The intramural hemorrhages and leucocytic infiltrations of the veins, so prominent in the controls, were also not seen in the heparin treated animals. It thus seems clear that in spite of the prominent inflammation at the prepared sites, including dilatation of the capillaries and venules and endothelial cell enlargement, significant hemorrhages failed to develop in the absence of thromboses in the heparin treated animals, which supports the concept of the fundamental importance of thromboses in the development of the local Shwartzman reaction. It may be significant to note that biopsies of control animals prepared in the same manner but in which provocative injections had not been followed by positive reactions showed an occasional thrombus in small veins. These were never observed in the prepared skin sites alone nor in the heparinized animals. This suggests that in animals in which the Shwartzman reaction was negative, thromboses had nevertheless occurred in small numbers following the provocative injection, but did not produce sufficiently widespread vascular occlusions to cause hemorrhage and infarction.

SUMMARY

Heparin administered at frequent intervals in large doses to rabbits completely inhibited the development of the local Shwartzman reaction. The same doses of heparin did not influence the pyrogenicity of the bacterial toxins, nor did it seem to alter the systemic manifestations of the toxicity of these materials. Histological studies show that the thromboses and hemorrhages so prominent in the early local Shwartzman reaction were inhibited by the heparin injections. The absence of hemorrhages in light of the essentially incoagulable blood seems to indicate that thrombosis is a fundamental process in the development of the local Shwartzman reaction. It is suggested that the anticoagulant activity of heparin is responsible for this inhibitory effect upon the local Shwartzman phenomenon.

ADDENDUM

Good and Thomas have recently reported similar, though more extensive, observations (5). They, too, have found that heparin in large doses completely inhibited the development of the focal dermal necrosis. In addition, they noted that heparin inhibited the renal necroses found in animals with systemic Shwartzman reactions. They also observed a failure of heparin to influence the leucopenic effect of the endotoxin and a failure of heparin to protect the animal from the lethality of the intravenous endotoxin injection. These observations are in complete accord with our own results.

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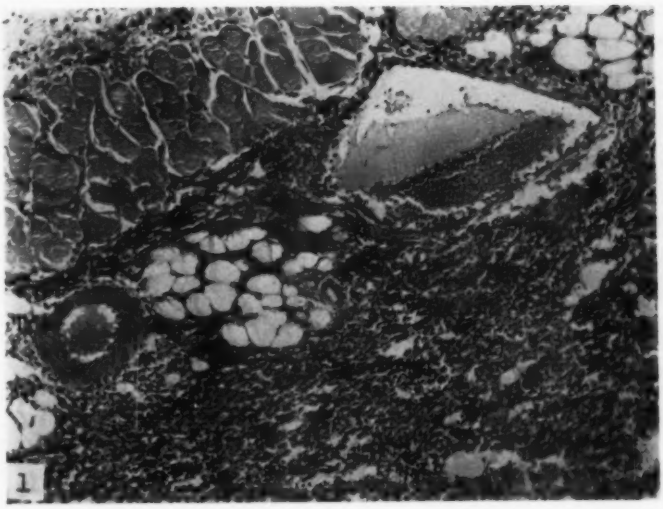
PLATE I

FIG. 1. Skin site 30 hours after preparation *before* intravenous provocative injection.

FIG. 2. Fresh thrombi in dilated veins in skin site of positive reaction 6 hours after provocative injection.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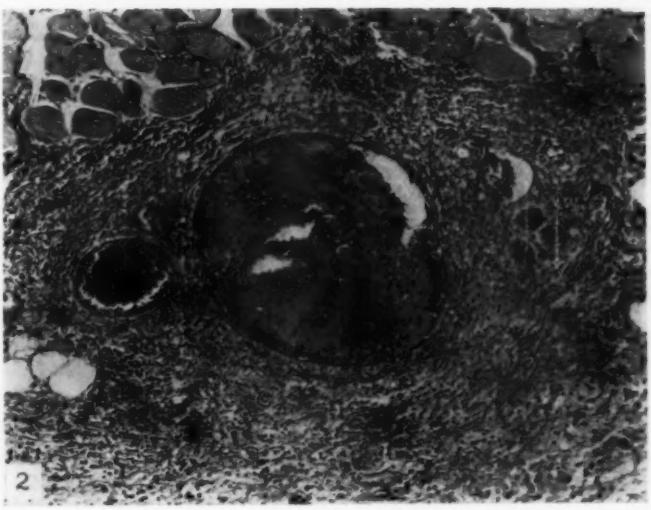
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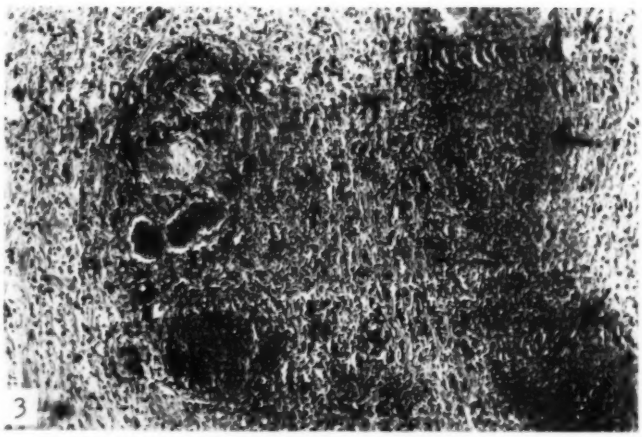
PLATE II

FIG. 3. Fibrin and leucocytic thrombi in veins with migration of leucocytes into perivascular tissues. Vein walls have been fragmented and separated by infiltrating red blood cells and leucocytes (arrows), 6 hours after provocative injection, in positive reaction.

FIG. 4. Fresh thrombus in dilated vein. Vein wall fragmented by hemorrhage and leucocytes (arrows), 6 hours after provocative injection, in positive reaction.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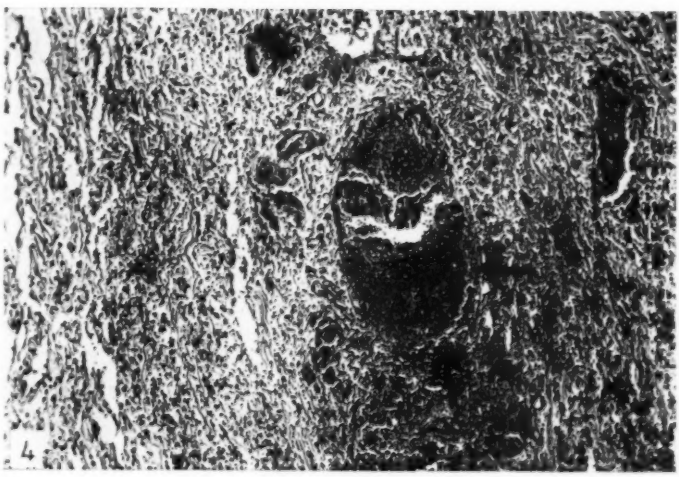
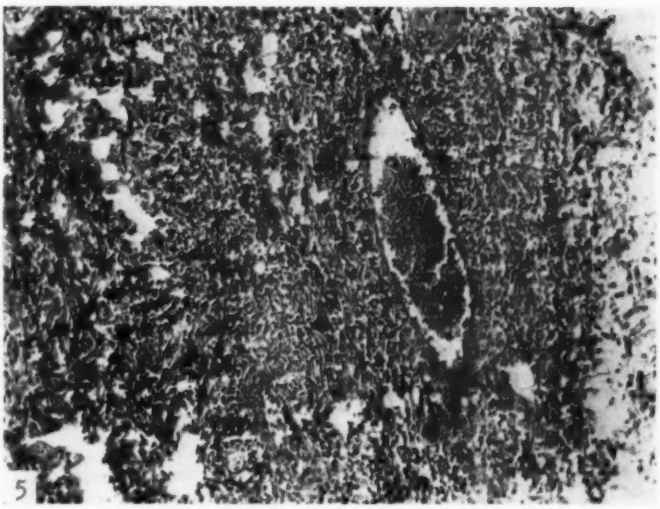

4

PLATE III

FIG. 5. Absence of thrombus formation or perivascular exudation in heparin treated animal, 6 hours after provocative injection.

FIG. 6. Same as Figure 5.



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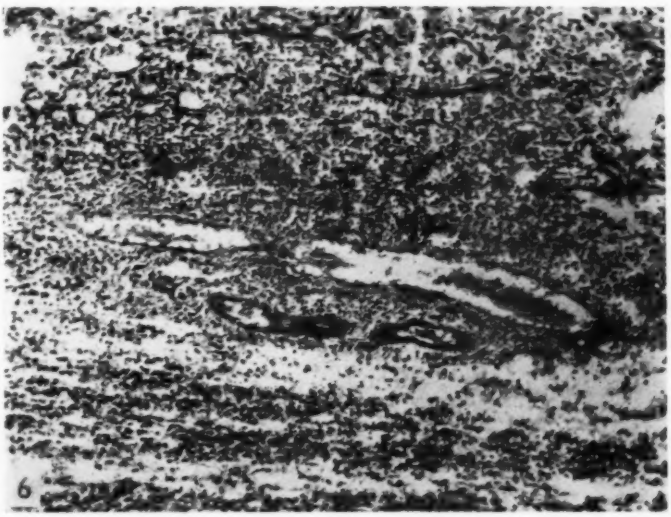

6

THE METABOLISM OF TISSUES FROM ANAPHYLACTICALLY HYPERSENSITIVE ANIMALS WHEN ANTIGEN IS ADDED IN VITRO[1, 2]

RICHARD H. FOLLIS, JR.

The Department of Pathology, The Johns Hopkins University School of Medicine

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It is generally agreed that two types of hypersensitivity may be recognized; these differ from one another in a number of respects (2). In one, the anaphylactic type, sensitization may be effected by microorganisms or simpler substances. Specific antibody can be demonstrated; passive transfer is possible. There is a prompt wheal and erythema with or without necrosis (Arthus reaction) when specific antigen is injected into the skin of the sensitive organism; a prompt generalized reaction (anaphylactic shock) can also be demonstrated. Available evidence (2, 3, 4) appears to indicate that the cellular sites of sensitivity are vascular endothelium and smooth muscle. The cells of certain tissues from hypersensitive organisms which have been studied *in vitro* in tissue culture do not appear to be damaged, at least as far as their ability to reproduce is concerned, by the presence of specific antibody in their nutrient medium (2, 5, 6).

In contrast, the second, or tuberculin, type of sensitivity is produced by whole organisms, living or dead; serum antibody cannot be demonstrated nor has passive transfer been effected as yet by serum from sensitive organisms. The skin reaction is one of delayed erythema with or without necrosis. A delayed generalized reaction may also be demonstrated. Tissue culture studies of certain cells from sensitized organisms have revealed evidence of injury when tuberculin is added to the nutrient medium (2).

At this time, we should like to present certain studies dealing with the anaphylactic type of sensitivity. Observations on the tuberculin sensitive state will be presented in a communication to follow.

MATERIALS AND METHODS

Male and female rabbits (averaging 2500 grams) and guinea pigs (averaging 500 grams) were sensitized by multiple injections of horse serum or crystalline egg albumin. Two to three weeks after the last sensitizing injection the animals were skin tested intracutaneously with the specific antigen in order to determine the state of hypersensitivity. Animals found not to

[1] Aided by grants from the John and Mary Markle Foundation and the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

[2] A part of these data was presented at a meeting of the Federation of the American Societies for Experimental Biology, Atlantic City, N. J., April, 1948 (1).

be sensitive were either discarded or given further courses of antigen. The uteri of the female guinea pigs were tested by the Dale technique at the time of sacrifice.

Tissues were obtained either after animals had been killed by a blow on the head or while alive under light ether anesthesia. The following tissues were studied: liver, spleen, kidney, testis, uterus, striated muscle and myocardium.

Slices were made with a razor blade, the tissue being held between two ground glass slides (8). They were weighed on a torsion balance and placed in Warburg flasks with vented sidearms. The main chamber of the flasks contained 3 cc. Krebs-Ringer-phosphate solution. One tenth cc. of 10% KOH and a square of fluted filter paper were placed in the center well and .2 cc. of the appropriate antigen in Krebs-Ringer-phosphate solution or a similiar amount of Krebs-Ringer-phosphate solution were placed in the sidearms. After oxygenation and temperature equilibration, initial readings were taken and the materials in the side arms were added. Readings were taken every hour for 3 to 4 hours. Q_{O_2} values are expressed as micro-liters O_2 removed per mgm. wet weight of tissue per hour, after correcting for flask constants. Ten per cent homogenates of tissues were prepared in water as described by Potter (7, 8). Measurement of succinic dehydrogenase and cytochrome oxidase activities were performed according to Potter's technique (7, 8). Tissues for homogenization were removed aseptically at operation so that each animal provided material for several observations.

RESULTS

Oxygen Consumption: Representative values for the Q_{O_2} per mgm. wet weight of various rabbit tissues are shown in Table I. No significant differences can be detected in O_2 consumption when the tissues from hypersensitive animals are studied in the medium with or without the addition of specific antibody.

Succinic Dehydrogenase Activity: Values of succinic dehydrogenase activity in terms of O_2 uptake per mgm. dry weight of various sensitized guinea pig tissues are shown in Table II. No significant differences are found in homogenates with and without the presence of specific antibody.

Cytochrome Oxidase Activity: Values for cytochrome oxidase activity in terms of O_2 uptake per mgm. dry weight are shown in Table III. No significant

TABLE I

Cumulative Oxygen Consumption (Averages in Microliters per Hour) of Tissue Slices from Rabbits Hypersensitive to Egg Albumin with and without Specific Antigen Added to the Media

TISSUE	NUMBER OF OBSERVATIONS	ANTIGEN ADDED			NO ANTIGEN ADDED		
		Hourly Period			Hourly Period		
		1	2	3	1	2	3
Heart	7	4.6	6.9	7.9	4.6	7.0	7.8
Liver	7	3.8	6.6	9.0	3.6	6.4	8.7
Kidney	7	10.4	19.4	27.6	10.0	18.8	26.9
Uterus	3	2.5	4.8	6.5	2.3	4.7	6.2
Spleen	4	4.8	8.5	12.0	4.2	7.9	11.6
Testis (teased tubules)	5	4.3	7.5	10.8	4.2	7.3	10.4

differences are found in the homogenates with and without the presence of specific antibody.

TABLE II

Succinic Dehydrogenase Activity (Averages in Microliters O_2) of Tissue Homogenates from Guinea Pigs Hypersensitive to Egg Albumin with and without Specific Antigen Added to the System

TISSUE	NUMBER OF OBSERVATIONS	ANTIGEN ADDED	NO ANTIGEN ADDED
Heart	5	96	98
Striated muscle	6	12	14
Uterine muscle	7	5	4
Liver	7	22	23
Kidney	6	35	34
Testis	4	5	4
Spleen	6	4	5

TABLE III

Cytochrome Oxidase Activity (Averages in Microliters O_2) of Tissue Homogenates from Guinea Pigs Hypersensitive to Egg Albumin with and without Specific Antibody Added to the System

TISSUE	NUMBER OF OBSERVATIONS	ANTIGEN ADDED	NO ANTIGEN ADDED
Heart	5	43	45
Striated muscle	6	32	34
Uterine muscle	7	38	36
Liver	7	26	24
Kidney	6	39	41
Testis	4	23	24
Spleen	6	56	57

DISCUSSION

From the characteristics of the anaphylactic type of hypersensitivity to which we have briefly alluded above, the only tissues which appear to be involved are smooth muscle (contraction) and vascular endothelium. It seems unnecessary to review in detail the evidence for this which, as already noted, is based on tissue culture studies of blood cells, splenic explants and liver cells (2, 5, 6), direct observations on blood vessels (3), histological studies on normal non-vascular or artificially vascularized rabbit corneas (4) and experimental serum disease in this animal (2). The contraction of smooth muscle *in vitro* was beautifully described by Dale (9) many years ago.

Under the conditions of the experiments described herein, it is, therefore, not surprising that derangement in metabolic activities of representative tissues from hypersensitive animals cannot be demonstrated *in vitro*, at least as far as oxygen consumption, or succinic dehydrogenase and cytochrome oxidase activities are concerned. It is likely that any increased oxygen con-

sumption occurring upon contraction of smooth muscle when antigen is added to the medium would be too small to be measured by the technique employed.

SUMMARY

1. When the oxygen consumptions of tissue slices from rabbits and guinea pigs sensitive to horse serum or egg albumin are measured in the presence and absence of specific antibody, no differences can be detected.

2. When the succinic dehydrogenase and cytochrome oxidase activities of tissue homogenates from rabbits and guinea pigs sensitive to horse serum or egg albumin are compared in the presence or absence of specific antibody no differences can be detected.

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MODIFICATION OF THE FERMENTATIVE ACTIVITIES OF PNEUMOCOCCUS THROUGH TRANSFORMATION REACTIONS*

ROBERT AUSTRIAN AND MARYDA S. COLOWICK

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Biological Division, Department of Medicine, The Johns Hopkins University School of Medicine, and the Department of Microbiology, New York University College of Medicine

Previous observations on the fermentative activities of pneumococcus (1) have shown that certain carbohydrates are fermented actively by all strains of this organism. There exists, however, a small group of carbohydrates which are fermented by some strains of pneumococcus but which appear not to be utilized by other strains under comparable conditions. The substances which fall into the latter category include the two glucosides, esculin and salicin, the polysaccharide, inulin, and the hexose alcohol, mannitol. In attempts to discover characters of pneumococcus the inheritance of which is controlled by transformation reactions, experiments were carried out to test the effect of the transforming principles from pneumococci which ferment actively one of the latter group of compounds upon the behavior of a seemingly inert strain toward the same substance. It has been found in the course of such studies that the apparent inability of certain strains of pneumococci to ferment salicin and inulin results from the conditions employed to test for such activity. In appropriate environmental surroundings, no strain of pneumococcus has been isolated which fails to ferment these two carbohydrates. Marked differences do exist, however, in the speed with which acid is produced when inulin or salicin is provided as the principle source of carbohydrate for different strains of pneumococcus growing under anaerobic conditions. The data to be reported concern the conditions favoring the utilization of inulin and salicin by certain strains of pneumococcus and the effect of the transforming principles of a rapidly fermenting strain upon a pneumococcus which produces acid slowly from salicin under anaerobic conditions.

MATERIALS AND METHODS

Fermentation Tests.—The tests were carried out in a liquid or semisolid medium containing 1 per cent of the carbohydrate to be tested. The liquid medium had the following composition:

Bacto beef extract	1 gm.
Bacto tryptose	10 gm.
Sodium chloride	5 gm.

* This work was supported by a grant-in-aid from the National Institutes of Health, Public Health Service, Federal Security Agency.

Dipotassium phosphate	1 gm.
Indicator	2 cc.
Distilled water	1000 cc.

All the ingredients save the indicator were dissolved in water by heating to 100°C, the beef extract being added last. If necessary, the medium was filtered through paper, and the pH adjusted to 7.4–7.5. The indicator was then added and the medium distributed as desired prior to autoclaving at 15 lbs. pressure for 20 minutes. The carbohydrate to be tested was then added to a final concentration of 1 per cent and the medium incubated for 24 hours to test for sterility.

The indicator used was a mixture of equal parts of 1.6 per cent brom cresol purple in 95 per cent alcohol and 1 per cent cresol red in 95 per cent alcohol (5 drops of 50 per cent sodium hydroxide were added to each 30 cc. of alcohol).

The semisolid medium was made by the addition of 2 grams of agar to a liter of the medium described above.

Solutions of inulin and salicin were sterilized by autoclaving at 10 lbs. pressure for 15 minutes. The inulin and salicin were obtained from the Baltimore Biological Laboratories.

Tests of the ability of pneumococci to grow in solid medium when various carbohydrates were provided in a concentration of 1 per cent as the principle energy source were carried out also in Cystine Trypticase Agar (Baltimore Biological Laboratories).

Enzymes.—Crystalline Desoxyribonuclease: 5 mgm. of crystalline bovine pancreatic desoxyribonuclease (Worthington Biochemical Sales Company lot D333) were dissolved in 10 cc. of charcoal-absorbed broth containing 0.02 M $MgSO_4$ and sterilized by passage through a Swinny filter. The solution was stored at 4°C and diluted immediately prior to use.

Crystalline catalase[1]: a sterile solution of recrystallized bovine liver catalase (Armour Laboratories lot No. R-491078) was used. This material had a potency of 390 Armour units per ml. and was diluted 1:5 prior to use.

Test for Peroxide Formation.—The technique employed was that described by Avery and Neill (2).

Preparation of Transforming Principle and Transformation Reactions.—The techniques employed were those described by MacLeod and Krauss (3).

Strains of Pneumococcus.—Five strains of pneumococcus manifesting inability to ferment salicin and/or inulin under the usual aerobic conditions of testing (1) were obtained in a lyophilized state from the State Serum Institute, Copenhagen, Denmark, through the courtesy of Dr. Erna Mørch-Lund.

Neufeld I: an encapsulated strain of pneumococcus type I fermenting inulin but not salicin.

Neufeld IR: an unencapsulated variant of pneumococcus Neufeld I manifesting the same fermentative activities as its parent strain.

I-519/43 41° x 300: an encapsulated strain of pneumococcus type I derived from the strain I-519/43 by 300 transfers at 41°C. The parent strain fermented salicin but not inulin in Hiss serum water but, after the treatment described, was unable to utilize salicin aerobically.

I41R: an unencapsulated pneumococcus obtained from the eighth transfer of strain I-519/43 41° x 300 in broth containing antibodies to type I polysaccharide. The unencapsulated strain was transferred eight additional times in type I anticapsular serum broth at

[1] Crystalline catalase used in these studies was supplied generously by Dr. K. C. Robbins, The Armour Laboratories and by Mr. C. C. Worthington, Worthington Biochemical Sales Co.

which time a single clone was selected for use in these experiments. This unencapsulated pneumococcus failed to ferment salicin and inulin in the liquid fermentation medium.

I41RS26 and I41RS36: two variants of strain I41R manifesting the ability to ferment salicin aerobically. These strains were obtained by serial daily transfer of progressively smaller inocula of strain I41R into fermentation medium containing salicin and incubated anaerobically. They represent isolates from the twenty-sixth and thirty-sixth transfers respectively.

Lederle II: an encapsulated strain of pneumococcus type II which does not ferment inulin or salicin aerobically in Hiss serum water.

VI B 2616/39: an encapsulated strain of pneumococcus type VI B which does not ferment inulin or salicin aerobically in Hiss serum water. It gives a positive quellung reaction with type VI pneumococcal typing serum[2].

R36NC: an unencapsulated pneumococcus derived from the encapsulated type II strain D39S and carried for many years in the laboratory. This strain ferments salicin and inulin rapidly under aerobic conditions.

EXPERIMENTAL

Effect of Cultural Conditions upon the Fermentative Activities of Pneumococcal Strains.—When pneumococcal strains Neufeld I, Neufeld IR, I-519/43 41° x 300, I41R, Lederle II and VI B 2616/39 were inoculated into liquid fermentation medium containing 1 per cent salicin and incubated aerobically they failed to ferment this glucoside. Similar results were observed with the strains reputedly unable to ferment inulin when this polysaccharide was substituted for salicin. If, however, 0.2 per cent agar was added to the same medium to make it semisolid, fermentation was observed to take place slowly in the lower portion of the aerobically incubated culture tube after a period of several days. This observation suggested that free access of atmospheric oxygen to the cells in the fermentation medium might interfere with their utilization of the available energy source, possibly by contributing to the formation of peroxides which have been shown to exert a deleterious effect upon certain carbohydrases of pneumococcus (4, 5). The inhibitory effect of peroxides was indicated further by the fact that all the pneumococcal strains listed above fermented both salicin and inulin when incubated anaerobically or when grown aerobically in liquid fermentation medium containing catalase (Tables I and II). Because pneumococcus strain I41R proved competent in capsular transformation reactions, it was selected for further study.

The behavior of pneumococcus I41R was compared with that of strains I41RS26 and R36NC under a variety of conditions. Unlike the former strain, the latter two ferment salicin with comparable speed both in aerobic and in anaerobic environments. When as small an inoculum as ten cells of either pneumococcus I41RS26 or R36NC was incubated aerobically in liquid fermentation medium containing 1 per cent salicin, the production of acid was detectable at

[2] The capsular typing serum used was made available through the courtesy of Dr. H. D. Piersma, Lederle Laboratories Division, American Cyanamid Company.

thirty-six hours. In contrast to the rapid production of acid under aerobic conditions of growth initiated by small inocula of these two strains, pneumococcus I41R failed to produce detectable amounts of acid from salicin unless inocula approximating 10^{10} cells were employed. Under ordinary conditions of testing

TABLE I

Fermentation of Salicin in Liquid Medium by Selected Strains of Pneumococcus under Various Environmental Conditions

STRAIN OF PNEUMOCOCCUS	AEROBIC		ANAEROBIC
	Catalase Absent	Catalase Present	Catalase Absent
I-519/43 41° x 300	−	+	+
I41R	−	+	+
I41RS36	+	+	+
Neufeld I	−	+	+
Neufeld IR	−	+	+
R36NC	+	+	+
Lederle II	−	+	+
VIB 2616/39	−	+	+

+ = acid formed; − = acid not formed.

TABLE II

Fermentation of Inulin in Liquid Medium by Selected Strains of Pneumococcus under Various Environmental Conditions

STRAIN OF PNEUMOCOCCUS	AEROBIC		ANAEROBIC
	Catalase Absent	Catalase Present	Catalase Absent
I-519/43 41° x 300	−	+	+
I41R	−	+	+
I41RS36	+	+	+
Neufeld I	±	+	+
Neufeld IR	±	+	+
R36NC	+	+	+
Lederle II	−	+	+
VIB 2616/39	−	+	+

+ = acid formed; − = acid not formed; ± = acid formation variable.

for fermentative activity, therefore, this strain would be considered to be unable to utilize salicin. When populations of pneumococcus I41R were incubated anaerobically in the same medium, however, acid formation resulted from initial seedings of ten to one hundred cells; but the production of acid took place at a very slow rate, six to eight days' incubation of the cultures being required for its detection. The slow growth and formation of acid from salicin by small inocula of strain I41R resulted apparently from the very slow adaptation of these organisms toward the utilization of the glucoside. When strain I41R was

plated in cystine-trypticase agar without added carbohydrate, it failed to give rise to visible colonies in this medium. Replicate platings in the same medium containing 1 per cent dextrose or salicin and incubated four days anaerobically, however, yielded essentially the same colony counts in the presence of either carbohydrate. This finding, together with the repeated demonstration of growth from an inoculum of fewer than one hundred cells in liquid medium in the presence of salicin as the principle energy source, provides strong presumptive evidence that strain I41R is able to utilize salicin as the result of a slow adaptive process rather than as the result of its being inert toward salicin and giving rise to actively fermenting mutants. When strain I41R was subcultured repeatedly under anaerobic conditions in fermentation broth containing 1 per cent salicin, the time required for the production of acid fell gradually, but ten or more transfers were required before the fermentative activity of the strain approached that of pneumococcus R36NC. It will be shown from the results of experiments reported below that the change in the fermentative activity of pneumococcus I41R subsequent to repeated cultivation in the presence of salicin is mutational. It appears, therefore, that pneumococcus I41R is able to utilize salicin but that its adaptive mechanisms are of such a nature as to render the utilization inefficient.

That the formation of peroxides may exert a deleterious effect upon the cellular components concerned with the splitting of salicin was suggested by the preliminary experiments recorded above. Further evidence to support this view has been obtained from the study of the formation of peroxides by pneumococcus strains I41R, I41RS26 and R36NC and from an examination of the fermentative activity of strain I41R grown under aerobic and anaerobic conditions in the presence and absence of crystalline catalase. When each of the three strains was cultured anaerobically in neopeptone broth from which the oxygen had been expelled previously by heating, no peroxide could be demonstrated in the culture by the benzidine test. If portions of the culture of any of these three strains were shaken in a flask containing air and allowed then to stand in the dark at room temperature, the formation of peroxide could be demonstrated in cultures of all three strains at the end of thirty minutes' aeration. The time required for the development of a positive benzidine test and the intensity of the color reactions indicated that the rates of peroxide formation by the strains fermenting salicin rapidly and the one fermenting salicin slowly were roughly comparable and that the ability to produce acid aerobically from the glucoside did not result from slow formation of peroxide by the rapidly fermenting strains. When strain I41R was grown anaerobically in the presence and absence of active crystalline catalase, no reduction in the time required for production of detectable amounts of acid was effected by the presence of the enzyme. Under aerobic conditions of incubation, however, the presence of catalase permitted fermentation to pro-

ceed as rapidly as under anaerobic conditions whereas detectable acid was not formed in the absence of the enzyme in aerobically incubated cultures.

Attempts to isolate an active salicinase from pneumococcus have been unsuccessful. It appears not unlikely, however, from the experiments of Neill and Avery (4, 5) and from the data presented here that the glucosidase

TABLE III

Reduction in the Time Required for the Production of Acid from Salicin by the Slowly Fermenting Pneumococcus I41R following Transformation with the Transforming Principles of the Rapidly Fermenting Pneumococcus R36NC

NUMBER OF CELLS INOCULATED	DAYS' INCUBATION ANAEROBICALLY						
	1	2	3	4	5	6	7
10^5		TNC		C			
10^4		TNC		C			
10^3		TNC				C	
10^2			TNC			C	

Symbols indicate initial day of detectable acid production by different sized inocula of pneumococcus I41R transformed with transforming principles of pneumococcus R36NC (TNC) and by similar inocula of control cultures (C). For various types of controls employed see text.

TABLE IV

Reduction in the Time Required for the Production of Acid from Salicin by the Slowly Fermenting Pneumococcus I41R following Transformation with the Transforming Principles of Its Rapidly Fermenting Variant, Pneumococcus I41RS26

NUMBER OF CELLS INOCULATED	DAYS' INCUBATION ANAEROBICALLY						
	1	2	3	4	5	6	7
10^7		TS26			C		
10^6			TS26		C		
10^5			TS26		C		
10^4			TS26			C	
10^3				TS26			

Symbols indicate initial day of detectable acid production as in Table III. TS26 = pneumococcus I41R transformed with transforming principles of strain I41RS26; C = controls.

splitting salicin is formed adaptively at markedly different rates by different strains of pneumococcus and that it is an enzyme inactivated by peroxides. This hypothesis would explain the failure of strains which produce the glucosidase slowly to ferment salicin under conditions which permit the formation of sufficient peroxide to inactivate the enzyme as it is formed.

Modification of the Fermentation of Salicin by Pneumococcus through Transformation.—The marked difference in the time required for the production of acid from salicin by strain I41R and by strains R36NC and I41RS26 prompted experiments to determine whether or not the production of acid by strain

I41R could be accelerated by transformation under the influence of transforming principles prepared from the more active strains R36NC, I41RS26 and I41RS36. When strain I41R was grown in the transforming system in the presence of the transforming principles of each of these three strains, a definite reduction in the time required for the production of acid from salicin was manifested by cells recovered following such exposure. Because a satisfactory technique was not evolved for detecting isolated clones developing from transformed cells, the behavior of unselected populations of strain I41R before and after transformation was studied. By this means a rough approximation of the number of transformed cells could be made. As indicated in Tables III and IV, tubes containing 1 per cent salicin in fermentation broth were inoculated with serial ten fold dilutions of the transformed and control cultures. Three types of control were used: strain I41R grown in the transforming system in the absence of any transforming principle, strain I41R grown in the presence of desoxyribonuclease and the transforming principles of a strain producing acid rapidly from salicin, and strain I41R grown in the presence of its own transforming principles. In all experiments, duplicate cultures of the test system and of the controls were examined. The results indicate clearly that the production of acid by strain I41R is accelerated after transformation in the presence of the transforming principles of the rapidly fermenting heterologous strain R36NC or of two of its own rapidly fermenting variants obtained by selective cultural techniques. The results suggest also that at least one per cent of the cells of strain I41R recovered following exposure to the transforming principles of strains producing acid rapidly from salicin had been transformed. There can be little doubt that the alteration was due to the transforming principles derived from the heterologous strain because no acceleration of acid production was observed in any of the three types of controls listed above. The inocula of transformed and control cultures used in the fermentation tubes were of comparable size and measurement of the rate of formation of lactate from glucose during growth by strain I41R before and after transformation reveals that alterations in this faculty were not responsible for the changes noted in the production of acid from salicin.

Despite the fact that transformation resulted in a decrease in the time required for the production of acid from salicin under anaerboic conditions by strain I41R, in none of the experiments was the alteration of cellular activity sufficient to lead to the production of acid under aerobic conditions of cultivation. Repeated transfer of populations of strain I41R in the presence of the transforming principles of the rapidly fermenting strain R36NC was carried out in an attempt to heighten the effect of the initial transformation but the results of a few such experiments have been irregular and inconclusive.

DISCUSSION

Examination of the fermentative activities of pneumococcus under a variety of cultural conditions indicates that certain differences between strains regarded as qualitative in the past may perhaps be quantitative in nature. Of the strains of pneumococcus failing to ferment salicin and inulin under the conventional conditions of testing, none examined has failed to ferment these two carbohydrates when the accumulation of peroxides during growth has been precluded. Such strains, however, produce acid very slowly from salicin under anaerobic conditions of growth, a finding which suggests that the rate of synthesis of an enzyme essential to the reaction is slow or that the total amount of enzyme made available to the reaction is small. The observation that the production of acid by such strains is inhibited under conditions of growth which permit the concomitant formation of peroxides is consistent with the findings of Neill and Avery (4, 5) that the carbohydrases of pneumococcus are inactivated readily by peroxide. If the glucosidase of pneumococcus hydrolyzing salicin manifests similar sensitivity to peroxide, the fermentative activity of a given strain should be determined by the relative quantities of enzyme and peroxide formed. Such an hypothesis would explain the apparent qualitative differences in the ability of some pneumococcal strains to ferment certain carbohydrates under the conventional methods of testing. Strains synthesizing large amounts of available glucosidase would hydrolyze salicin even in the presence of peroxide whereas strains forming small amounts of available enzyme would appear inert as a result of inactivation of the small quantity of enzyme by peroxide. In the absence of satisfactory techniques for isolating and quantifying the appropriate enzyme, however, it is not possible to be certain that variations in the amount of enzyme formed rather than some alternative mechanism is responsible for the observed differences in the behavior of pneumococcal strains.

It is of considerable interest that the production of acid from salicin by pneumococci may vary in the direction of either increased or decreased capacity. Several strains of pneumococcus studied in these laboratories as well as strain I-519/43 41° x 300 have manifested a sufficiently marked reduction in their ability to ferment salicin following cultivation on laboratory media that they are unable to utilize this glucoside under the conventional aerobic conditions of testing. On repeated transfer in a selective cultural environment in which salicin is provided as the principle energy source, such strains acquire again the ability to ferment salicin in environments permitting the concomitant formation of peroxides. These variations in fermentative activity may be analogous to the quantitatively variable behavior of several other pneumococcal characters: capsular polysaccharide synthesis (6, 7) and resistance to penicillin (8). The spontaneous acquisition by a previously inert

strain of the ability to produce acid from salicin under conditions permitting the formation and accumulation of peroxides is evidently a mutational rather than an adaptive change. This conclusion is borne out by the observation that transformation of the parent inert strain with the transforming principles of its active offspring results in acceleration of the production of acid from salicin by the transformed cells. The results are in several respects analogous to those reported by Hotchkiss (8) regarding the acquisition of resistance to penicillin by pneumococci through transformation. In these experiments, a resistant mutant was derived from a previously sensitive strain by the use of a selective cultural technique. The sensitive parent strain was then transformed to a state of heightened resistance to penicillin under the influence of the desoxyribonucleates of the selected resistant mutant. In no instance, however, was the resistance to penicillin acquired by the sensitive parent strain through transformation equal to that achieved through selection. The results of transformations involving time required for the production of acid from salicin are similar in this respect. They add another to the growing list of pneumococcal characters which are subject to heritable alteration through transformation reactions.

SUMMARY

The fermentation of salicin and of inulin by selected strains of pneumococcus has been demonstrated to be influenced by environmental conditions. Strains which ferment these carbohydrates slowly under anaerobic conditions of growth fail to produce acid from them in environments permitting the formation and accumulation of peroxides. The formation of acid from salicin by one such slowly fermenting strain has been shown to be accelerated following exposure to transforming principles prepared from two selected mutant strains and one heterologous strain, all of which ferment salicin rapidly under aerobic conditions permitting the accumulation of peroxides.

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DESTRUCTION OF A HUMAN FIBROSARCOMA STRAIN (A.Fi.) IN TISSUE CULTURES FOLLOWING THE GROWTH OF POLIOMYELITIS VIRUSES

A Preliminary Report*

M. STOLER AND M. K. GEY

Departments of Medicine and Surgery, The Johns Hopkins University School of Medicine

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The recent demonstration that poliomyelitis virus will grow in and destroy human and monkey embryonic non-neural tissues (1) has stimulated a new interest in this virus. A spate of papers on the growth of this virus in tissue culture has ensued. However, useful as this technique is, the factors influencing a particular cell to produce virus cannot be adequately studied in primary explants since these consist of mixed cultures of cells. Such studies on the host-cell–parasite relationship are much better performed in systems where uniform cell strains are demonstrated to be susceptible to a particular virus (2). The susceptibility of certain rat and human cell strains to the virus of equine encephalomyelitis (3) led us to test several human cell strains for their susceptibility to poliomyelitis virus.

We have found that a human fibrosarcoma strain (A.Fi.) which has been grown in continuous tissue culture for fifteen years, is susceptible to all three strains of poliomyelitis virus. The Lansing strain of virus has furthermore been serially transferred to new cultures ten times, each time causing infection and destruction.

MATERIALS AND METHODS

Roller tube cultures (4) of strain A.Fi., which had been maintained by M. K. Gey for the most part in a composite plasmatic medium previously described (5), were transferred to 25 per cent chicken serum in balanced salt solution (Gey). Penicillin (100 units) and streptomycin (10 units) were added also. Under the conditions provided the cells grew directly on the glass wall. The use of this serum solution assured no carry-over of antibody from human serum sources. The cultures were maintained at 34°C. Cultures with a good migratory zone of cells, following two to three days of incubation were depleted of their medium and treated with a virus suspension and new fluid and then incubated further at 34°C. The Lansing infection (courtesy Dr. D. Bodian) was initiated with the supernatant from a 20 per cent brain emulsion from paralyzed mice. This suspension titered to 10^{-2}. The virus was transferred to new roller tube cultures by inoculating freshly prepared cultures with two drops of supernatant fluid from cultures which had been destroyed by the virus. The susceptibility of this cell strain (A.Fi.) to the other strains of poliomyelitis virus

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was tested by inoculating appropriate roller tube cultures with the first tissue culture passage of the Leon and Brunhilde strains. These had been grown in human embryo skin and muscle by Dr. J. F. Enders, who kindly furnished us with the fluid from first passage cultures. All cultures were examined daily, and the effects of the virus noted. The phase microscope pictures here recorded were made from cultures set up separately in 2″ x 3″ drilled slide chambers. (See explanation of plates.)

Although this cell line grows indefinitely in human placental cord serum (40 per cent solution) and initially in dilute chicken serum, in both a moderate number of cells may be found destroyed throughout the tube. This occurs in the absence of known infection and is reduced by incubation of the cultures at 34°C instead of 37°C.

RESULTS

The inoculation of the brain suspension was followed one day later by a beginning fraying of the borders of the expanded cells. This progressed to the point where a large proportion of the cells became rounded, dark, granular, and irregular in outline. After 60 hours, most of the cells were destroyed and

TABLE I

Neutralization of Cytolytic Effect by Immune Serum

	DILUTION					
	1:10		1:50		1:250	
Normal	+++	+++	+++	+++	+++	+++
Immune	0	0	0	0	0	0

cytolyzed fragments and debris were scattered throughout the tube. Figure 1 shows the typical effect of the virus two days after inoculation. The destruction produced by the Leon and Brunhilde strain was similar in appearance but occurred earlier. However, this does not take into account the higher titer of the inoculum of the latter strains.

The destructive effect of the Lansing virus was observed throughout the ten passages. It was uniformly complete 72 hours after virus was added. The supernatant fluid from the cultures showed a low titer on reinoculation of mice, varying from $10^{-1.4}$ to $10^{-1.8}$ in the fourth and sixth passage.

Neutralization of the cytolytic effect of the virus was studied by combining supernatant fluid with immune sera. Various dilutions of the immune monkey serum and of a control normal monkey serum were combined with equal volumes of a 1:10 dilution of the supernatant fluid from the seventh culture tube. The mixtures were kept at room temperature (22°C) for one-half hour. Two drops of each dilution were inoculated into two new cultures of A.Fi., and these were observed for changes during the next three days. Neutralization was complete in all the dilutions tested (Table I), and all of the cultures inoculated with virus and normal serum were destroyed.

SUMMARY

The successful passage of the Lansing strain of human poliomyelitis through ten passages in roller tube cultures of a human tumor cell strain (A.Fi.) is reported. Evidence for this rests on its uniform destructive effect in all passages, on the recovery of the virus in mice and the neutralization of the destructive effect by immune monkey serum. The process of destruction has been studied by phase microscopy. The other strains (Leon and Brunhilde) also destroyed these cells, but tissue culture passage was not carried out.

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PLATE I

FIG. 1. Roller tube culture of human fibrosarcoma (A.Fi.). Growth of cells after transfer to 25 per cent chicken serum and incubated at 34°C. ×130.

FIG. 2. Destruction of cells by Lansing strain of poliomyelitis. Roller tube culture of A.Fi. in 25 per cent chicken serum 2 days after addition of virus ×130.

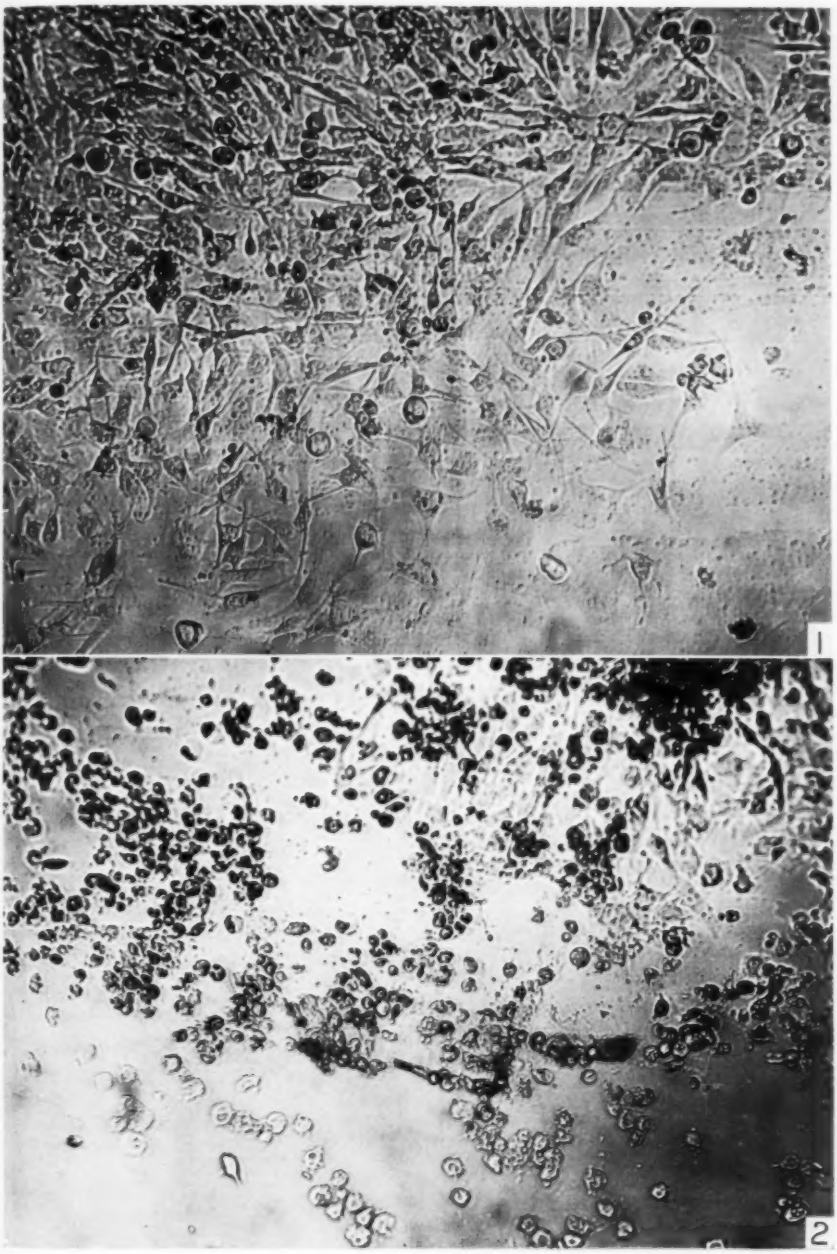
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PLATE II

PHASE MICROSCOPE PICTURES OF DESTRUCTION BY POLIOMYELITIS VIRUS

FIG. 3. Uninfected explant of colony of human fibrosarcoma (A.Fi.) transplanted into 25 per cent chicken serum. Three days old. ×700.

FIG. 4. Infected cells from explant of A.Fi. set up three days previously. Virus added 2 days previously. Irregular fraying of edges progresses to Figure 7. ×700.

FIG. 5. Similar culture, but area showing greater destruction. Cells ballooned and disintegrating. Nucleus present but not visible. ×700.

(Photographs by Mr. Peter Sapranauskas with American Optical Company Phase Microscope, 4 mm. objective Dark M.) All phase pictures were taken in specially designed chambers (3).

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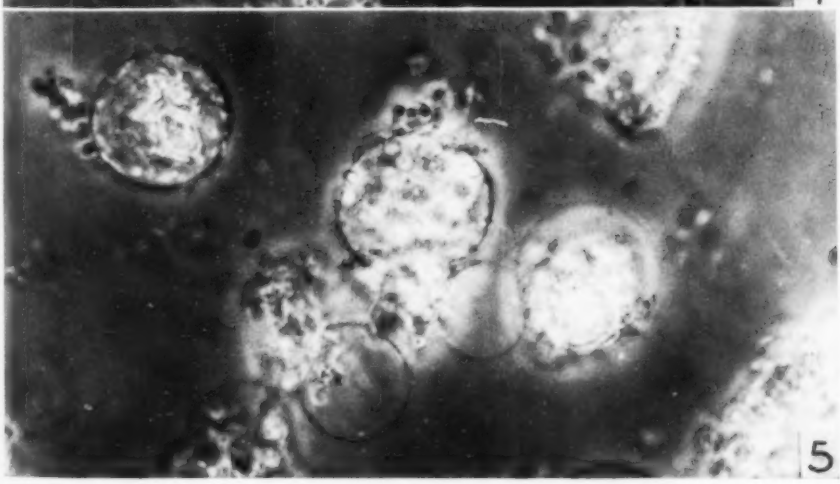
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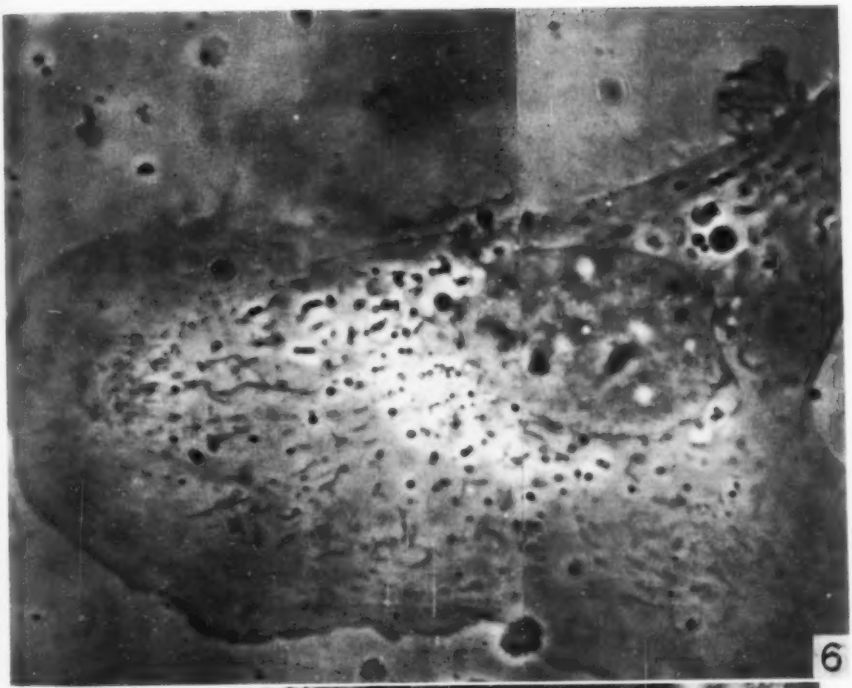
PLATE III

FIG. 6. Normal extended cell from uninfected 3-day culture of A.Fi. Nucleus in center, fat droplets and mitochondria clearly outlined. ×2000.

FIG. 7. Infected cell from culture of similar age. This destroyed cell shows the typical destruction which occurs after two days of infection. Motion pictures of this culture showed that the destructive processes progressing from that shown in Figure 4 to that in this figure takes as long as 12 hours. ×2000.

Virus was recovered from all infected cultures by intracerebral inoculation of the culture fluid into mice. Control cultures, observed as late as two days after these pictures of infected cultures were obtained, showed the great majority of the cells in a healthy expanded state.

(Photographs by Mr. Peter Sapranauskas with American Optical Company Phase Microscope, 1.8 mm. objective Dark M.)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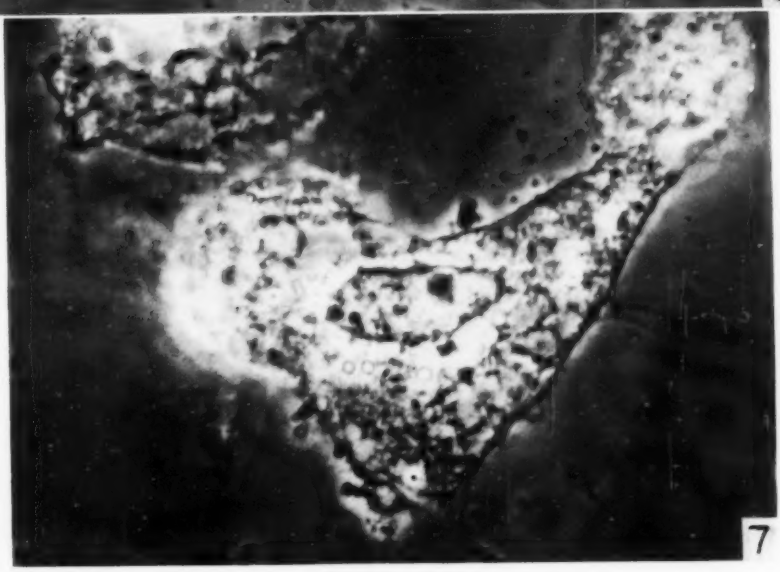
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MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

Hurd Hall, February 9, 1953

Dr. Jonas Friedenwald: The meeting will please come to order. The first paper is by Drs. Palmer H. Futcher and Gaston Sauve. It will be presented by Dr. Futcher.

DETECTION OF MILD DIABETES MELLITUS BY FEEDING GLUCOSE TO INDUCE GLYCOSURIA

An attempt has been made to discover mild diabetes mellitus by feeding glucose to induce glycosuria. Studies were made of 1000 ambulatory patients not previously suspected of suffering from diabetes mellitus; with rare exceptions the subjects were over 40 years of age. The patients had applied to the Private Out-Patient Service of the Johns Hopkins Hospital for diagnostic study and presented a variety of complaints. The urine of 191 of these patients, voided two and one half hours after the ingestion of 75 gm. of glucose, reduced Benedict's solution. Additional studies, including glucose tolerance tests, sufficient to elucidate the possible presence of an impairment of glucose metabolism, were performed on 70 of these 191 subjects. In 31 of these 191 subjects there were demonstrated varying degrees of impairment of carbohydrate metabolism which would not have been recognized had the studies been limited to examination of the fasting urine and measurement of the fasting blood sugar.

This report will appear in *Diabetes*, January–February issue, 1953.

Dr. Friedenwald: Is there any discussion of this paper?

Dr. Henry M. Thomas, Jr.: I would like to ask Dr. Futcher a question or two. Not being a statistician, I am not clear about the incidence of positive results. Wouldn't this 3% more properly be raised to about 8% if you adjusted it for the 121 patients that you didn't study? You studied only 70 of the 191 patients that were positive. So, you might have had considerably more diabetics discovered by your screening test. It seems to me that this is extremely important and this simple test that Dr. Futcher has carried out has brought to light a very significant group of cases. The false positive ones are not too surprising in that in the last war a great many soldiers were discovered to have glycosuria and were studied very carefully. Most of them were found to fall into the group of simple alimentary glycosuria, or into the group of individuals with a low renal threshold for sugar. So, I would think that this group of false positives still must be studied, and it is certainly nothing against this test. I would like to ask Dr. Futcher if he has enough experience to tell us whether the abbreviated two-hour glucose tolerance test has given results that he thinks are adequate in determining whether the patient falls in the false positive group or in the significant group.

Dr. Friedenwald: Any further discussion?

Dr. Futcher: Dr. Thomas asked as to the influence upon the overall statistics on the incidence of impaired carbohydrate metabolism which might have been exerted if we had performed glucose tolerance tests on the 121 unstudied patients with positive screening tests. It certainly seems reasonable that if we had carefully studied them, there would have been cases from that group which would have raised our 5% incidence of otherwise unrecognized

diabetes or impaired carbohydrate metabolism in the 1000 subjects to a higher figure. I should emphasize, however, that there was a natural tendency to select for glucose tolerance tests those patients who showed the heavier grades of glycosuria during the screening test. So the incidence of impaired metabolism in those 121 unstudied patients might be somewhat lower than in those whom we did study. A word as to my suggestion of the use of an abbreviated two hour glucose tolerance test in determining whether there is impairment of carbohydrate metabolism in those manifesting a positive screening test. There is a general tendency to interpret the results of the standard three hour glucose tolerance test on the basis of the two hour level, paying little attention to the peak at one-half or one hour but looking to see whether the blood glucose concentration comes down below 120 mgm. per cent in two hours. The standards of the American Diabetes Association, which may be a little too rigorous, state that, in a normal glucose tolerance test, the blood glucose concentration falls below 120 mgm. per cent in two hours. Some people think this is a little arbitrary and would say that if it is above 140 mgm. per cent at two hours, it is certainly abnormal. If it is between 120 and 140 mgm., we consider the impairment merely questionable. In that regard, we are somewhat careful of what we tell patients whom we suspect of having these admittedly mild degrees of impairment of carbohydrate metabolism. If they have fallen in this group of minimal impairment, we tell them that, on the basis of this single glucose tolerance test, we have found evidence of mild impairment. We advise them to be followed every six months to a year as far as their urinary sugar goes. We tell those who are overweight that it is advisable that they lose weight and come back to normal. We do not tell them that they have diabetes mellitus, but we merely mention the possible impairment on the basis of the one test. To those who have a family history of diabetes, we are a little bit more vehement in our warnings.

Dr. Friedenwald: The next paper is by our new professor of chemistry, Dr. Albert L. Lehninger. I do not think this is an occasion for formal introduction and can only say that we are all very happy to have such a distinguished successor to the chair to which our old and still young friend, Bill Clark, brought so much luster. I hope that Dr. Lehninger's participation at this meeting tonight is the beginning of a long and frequent attendance at our meetings and participation in our programs.

THE BIOCHEMICAL ORGANIZATION OF MITOCHONDRIA

With the development of methods for the isolation of nuclei, mitochondria, microsomes and other particulate elements of the cell from tissue dispersions by differential centrifugation, it has been possible to examine the intracellular localization of some important enzymes and enzyme systems. The use of isotonic sucrose solution as dispersing medium has permitted isolation of mitochondria from a number of tissues in "native" condition, showing the morphology and vital staining characteristics given by mitochondria in unfixed sections. Such preparations when supplemented with substrate, Mg^{++}, adenine nucleotide, K^{+}, and orthophosphate will catalyze the oxidation of all the intermediates of the Krebs tricarboxylic acid cycle, the oxidation of fatty acids, and the accompanying oxidative phosphorylations. The mitochondria are the only cellular components capable of these highly organized biochemical functions and the mitochondria can easily account for the known rates of respiration of the intact tissue.

These highly organized enzyme systems, composed of many individual enzymes functioning together in a highly integrated manner, are highly dependent on the structural integrity of the mitochondrion for organized enzymatic activity. Mitochondria isolated from a number of normal tissues of higher animals, experimental malignant tumors, from higher

plant cells and microorganisms have been found to catalyze organized biological oxidations and phosphorylations. In addition to functioning as the "power plants" of the cell, the mitochondria also appear to be sites of very active synthesis of phospholipids and pentose-nucleic acids. It appears doubtful from recent work, however, that the mitochondria are important sites of protein synthesis.

The mitochondrial membrane, which is easily visualized by electron microscopy, has semipermeable characteristics. This membrane is freely permeable to K^+, Na^+, Cl^-, $HPO_4^=$ succinate, Ca^{++} and other substances but is impermeable to sucrose, hexoses and hexitols, and a wide variety of phosphate esters, including adenine nucleotides. These permeability characteristics affect the metabolic exchange between the mitochondria and the soluble portion of the cytoplasm. Evidence exists that closely related substances may compete for entry into the mitochondrion.

Isolated mitochondria are capable of concentrating a number of substances against concentration gradients but only during active phosphorylating oxidation. These substances include Mg^{++}, K^+, Na^+ H^+, $HPO_4^=$, and fumarate. Such findings indicate a possible secretory function for mitochondria.

Certain of the enzymes of electron transport appear to be embedded in the structural matrix of the mitochondria and in this form have much higher catalytic activity than in true solution, indicating favorable spatial juxtaposition of active groups in the matrix. Experimental approaches are now at hand for studying the intramitochondrial localization of certain respiratory enzymes.

Mitochondria isolated from experimental tumors also possess highly organized respiratory activity but in general differ from those isolated from normal tissues in being far more permeable and "leaky". This property causes the appearance of certain gross differences in phosphorylating activity which may possibly be of functional significance.

Dr. Friedenwald: Are there any comments or questions?

Dr. Arnold R. Rich: I would like to say that I think that Dr. Lehninger's exposition of the problems in this highly important realm has been one of the simplest, clearest and most delightful imaginable. I would like to ask you, Dr. Lehninger, in relation to the mitochondrial enzymes responsible for each of the separate reaction-steps that lead to a given end product, whether you believe that each enzyme involved is a separate and distinct enzyme protein arranged spatially in the appropriate juxtaposition, or whether it is possible that several may be specific, active surface groupings arranged in a convenient order on a single large protein molecule?

Dr. Lehninger: Well, I should say, that although there is some speculation about multi-headed protein molecules, actually a good many of the enzymes involved in these complex patterns which I have described have been isolated, and some brought to a very high degree of purity. I think there is little reason to doubt that they are actually proteins of individual specificity. On the other hand, I think one might well consider that the matrix of the mitochondrial body, in which these enzymes are presumably embedded, could be regarded as a sort of macro-molecule, very highly adapted to the situation in a structural sense.

Dr. George Gey: I have had the pleasure of studying living mitochondria in many types of cells, and I can support in part what Dr. Lehninger said about them. On the basis of motion pictures and other studies that have been carried out, we have observed these living mitochondria to be propelled throughout the cells along various complex pathways, by a kind of

streaming cyclosis which is universal in the cells. There are times when mitochondria change in their morphological aspects. One can see lateral buds protrude perpendicular to the linear aspects, indicating there there are times when the membrane which surrounds the mitochondrion can suddenly change. The first interpretation of these observations was that some fibrous components within the cell might have attached themselves to the membrane and pulled it out. In connection with your statements regarding differences in normal and malignant cell mitochondria, especially as to numbers, I would say that my own observations are at great variance to the general rule that you stated. We have strains of malignant cells with a few mitochondria, and we have other strains of malignant cells with a great many mitochondria, so many, that they rather spoil the picture when one is trying to get a reasonable view of what is going on. I wondered whether in your statement regarding the differences between normal cells and malignant cells you wouldn't be willing to venture a statement, if the information is available, on the permeabilities of mitochondria from regenerating cells and embryonic cells. There is an old dictum that has been handed down for many years that malignant cells are in many respects like embryonic cells. This is a very important point to keep in mind in our concepts of differences between normal and malignant cells.

Dr. Lehninger: In the first place, in respect to something brought up about the movement of mitrochondria, through the cytoplasm, the earlier literature on this subject has always been rather absorbing to me. Kingsbury once described pathways taken by a single mitochondrion in unfixed sections, which showed that the mitochondrion made a number of round trips between the nucleus and the cell wall.

Dr. Gey: As far as I know, no one has observed this before Dr. Lewis observed it. We have very excellent motion pictures showing to-and-fro movement from the plasma cell membrane with a regular shift in travel, back and forth, along very definitive pathways.

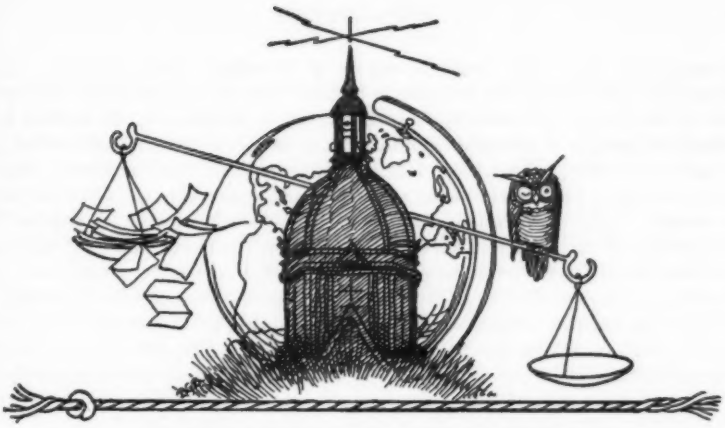
Dr. Lehninger: Have you observed motility in isolated mitochondria?

Dr. Gey: I don't know whether there has ever been reported any motility on the part of the isolated mitochondria. The movement seems to be completely passive.

Dr. Lehninger: In respect to the question of the sizes and the number of the mitochondria in the tumor cells, I meant to say that the decreased numbers of mitochondria were observed in the particular tumors we studied but I am of course aware that no generalization can be made. With respect to the question of similarity of mitochondria from malignant cells compared to embryonic mitochondria and to mitochondria in regenerating tissue, to my knowledge, no one has done much in the line of examining the question of their relative permeability characteristics. This question is a rather new area of research, and I don't think there has been a great deal of work on it. Certainly, it would be an obvious thing to look into.

Dr. Friedenwald: Any other comments? If not, I want to thank you very much. The meeting is adjourned.

(The meeting was adjourned at 10 p.m.).



BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

The Radiant Universe. By GEORGE W. HILL. 489 pp., $4.75. Philosophical Library, New York.

The author proposes to explain all phenomena in terms of "Basic Radiation". "This radiation cannot be isolated as it permeates all space, all matter and is the reservoir from which all energy is drawn and to which all energy is eventually returned. All else is but manifestation of change in this energy."

In pursuing his exposition the author is in contradictions with practically all principles of physics although often it appears superficially as if he made use of some of the concepts of modern physics. Unorthodox views may be of course quite refreshing and lead to new facts or at least a new outlook. The present book deals with practically the whole universe. The treatment is qualitative and the avoidance of any quantitative treatment allows the author only to draw vague conclusions. One has the impression that he gained his knowledge of the facts only from the popular books and accordingly he quite often misunderstands their meaning. Numerous examples might be quoted. The author makes much of the fact that his theory requires that "the instantaneity of transmission of the gravitational influence must be denied". Actually a finite velocity for the propagation of gravitational effects has been taken for granted ever since Einstein's general theory of relativity.

The problem of the structure of the universe has always attracted much attention and in recent years tremendous progress has been made toward extending our knowledge. In the reviewer's opinion the present book is not one where an account of this progress can be found. It represents the personal views of the author and it is doubtful whether they are shared by any informed person.

G. H. DIEKE

Die Zerebrale Angiographie. By H. KRAYENBUHL AND R. RICHTER. 217 pp., Ganzl. DM 59.70. Georg Thieme Verlag, Stuttgart, Germany.

The authors of this monograph are neurosurgeons in a large medical center and the methods and materials presented represent their experience in the performance of more than

1500 angiograms since 1936. Nearly all aspects of the subject receive attention. The development of cerebral angiography briefly is followed from its introduction in 1927 by Egas Moniz to the present. The technique of angiography as described by the authors is conventional and consists in the serial exposure of three films so as to record the arterial, capillary and venous filling phases. Both lateral stereoscopic and anterior posterior films are routinely obtained; additional oblique projections are occasionally employed so that the total amount of contrast substance (35% Nosydrast or Diodrast) is of the order of 35–50 cc. per patient. There is no mention of the incidence of significant untoward reactions in the authors' series but one would judge that they were not excessive although the amounts of contrast substance used are large. Both lateral stereoscopic views admittedly are desirable but their value must be weighed against the potential dangers of damage to the cerebral vascular system from large amounts of contrast substance. The authors rightfully place considerable emphasis on good venograms but with only three films one wonders how often they failed to obtain such. However, the numerous reproductions of venograms throughout the book would indicate that they were a constant feature of their examinations.

The normal anatomy of the blood vessels of the brain and scalp is covered adequately, but briefly and always in relationship to the roentgenographic appearance. Minor normal variations are not extensively considered and this is probably desirable since some of the variations are hardly perceptible in the angiograms and an extensive description is not warranted. The venous system and the posterior group of vessels supplied by the vertebral arteries receive adequate consideration and there is a good discussion of the collateral circulation of the brain and scalp. The consideration of the physiology of the cranial vessels is limited to those factors the effects of which can be observed from the angiogram and the subject is sufficiently covered from a clinical point of view.

The reviewer is in entire agreement with the discussion of the dangers of cerebral angiography and most of the complications of the procedure are mentioned by the author. However, the incidence of untoward major reactions in the author's series is not precisely stated. Also there is scant reference to radiation hazards to the operator and method of protecting against same. This serves to strengthen our impression that most non-radiologists tend to underestimate this danger.

In connection with the abnormal angiogram there are excellent sections on vascular diseases of the brain and skull; the localization and typing of space-occupying intracranial processes; hydrocephaly and injuries of the brain and skull.

The *pièce de résistance* of the entire work is an atlas of cerebral angiography. This atlas is composed of a clinical and roentgenological consideration of 71 cases of intracranial abnormalities, all but two or three of which were confirmed by operative or autopsy studies. Case histories, neurological examinations and occasional air studies are all brought together for consideration with the pertinent angiograms. Reproductions of films are positives, but are quite clear and are usually accompanied by adequately labelled line drawings and sketches. Also accompanying each case there is a short discussion of the abnormalities visible on each reproduction and its relationship to the final diagnosis. This is a storehouse of information of great practical value.

The entire volume follows the usual high level of reproductions and illustrations set by German and Swiss authors and publishers. The paper is of high quality and the book is well bound. There is a bibliography containing about 175 entries and the index is adequate. The book is a welcome and significant contribution and should be sought by both neurosurgeons and radiologists.

ROBERT N. COOLEY

Practical Dermatology. For Medical Students and General Practitioners. By GEORGE M. LEWIS. 328 pp., $7.50. W. B. Saunders Co., Philadelphia, Pa.

This book measures ten, by six and a half, by three quarter inches, contains three hundred and twenty-eight pages, and therefore is, as a representative of dermatological texts, quite small.

The subject matter is encompassed in twenty-five chapters, each with a clearly understandable title. It is fully illustrated with excellent photographs from the author's collection. An attempt is made to group in each separate chapter diseases which are related, either from the symptomatic, causative or pathological standpoint. For example Acne Vulgaris, Rosacea and Seborrhoeic Dermatitis are described in the second chapter, the so-called collagen diseases in the sixth, and malignant tumors in the twenty-third. The entire twenty-fifth chapter is devoted to principles and methods of treatment. The discussion of topical medication is especially good.

The commoner diseases are presented in rather full detail, the rarer ones in synopsis form. One very common dermatological problem, however, was unfortunately almost entirely overlooked, namely the eczematous eruptions affecting the hands. Regarding many diseases, present day concepts of etiology and likewise very new treatment methods are brought out in a textbook for the first time. The uses and abuses of ACTH and cortisone are included. No source references in the *corpus* are made, but a bibliography is appended. Histological descriptions are purposely omitted for brevity.

H. HANFORD HOPKINS

Endocrine Treatment in General Practice. Edited by MAX A. GOLDZIEHER AND JOSEPH W. GOLDZIEHER. 488 pp., $8.00. Springer Publishing Company, Inc., New York, New York.

Since knowledge of the functions of the endocrine glands has advanced so rapidly in recent years, there are very few textbooks which provide the practicing physician with adequate information on current concepts of the management of endocrine disorders and the use of hormonal substances therapeutically. This book has been designed to supply a quick source of reference for the application of hormonal preparations in various disease states.

With this purpose in mind, there are several chapters of value, such as those on carbohydrate and fat metabolism, water and electrolyte metabolism, male infertility, and the menopausal syndrome. Many chapters deal with disorders of organ systems; consequently there is frequent repetition of various topics, *e.g.* the treatments of adrenal insufficiency and the menopause are discussed in several different sections of the book.

The editors state in the introduction that it was unnecessary to include discussions of controversial opinions. Instead, however, there are dogmatic statements made on types of treatment for which there is little rational basis and which are extremely controversial even among endocrinologists. For instance desiccated thyroid and anterior and posterior pituitary extracts are recommended as adjuncts in the management of obesity. Many endocrinologists would not agree on such usage of these hormonal substances. Desiccated thyroid particularly is advocated in many conditions in which there is no proof that it has beneficial effects.

ACTH and cortisone have been proven valuable in the alleviation of many disorders. Too little particular attention, however, is given in many sections of the book to the deleterious effects of overdosage or of sudden withdrawal of these powerful hormones. It is also not sufficiently stressed that the administration of ACTH or cortisone produces temporary amelioration but not permanent cure of pathological states.

This reviewer believes that the book will tend to give the general medical practitioner a false sense of security and to cause him to rely too extensively on hormones in therapy to the exclusion of other well established modes of treatment.

J. G. W.

BOOKS RECEIVED FOR REVIEW

American Pocket Medical Dictionary 19th edition. 639 pp., $3.25 plain; $3.72 with thumb-index. W. B. Saunders Co., Philadelphia, Pa.

The Anatomy of the Nervous System. Its Development and Function 9th edition. By STEPHEN WALTER RANSON. Revised by SAM LILLARD CLARK. 581 pp., $8.50. W. B. Saunders Co., Philadelphia, Pa.

The Autonomic Nervous System, 3rd edition. By JAMES C. WHITE, REGINALD H. SMITHWICK AND FIORINDO A. SIMEONE. 569 pp., $12.00. The Macmillan Co., New York.

Diseases of the Heart and Arteries. Anatomical and Functional Disturbances of the Circulation. Treatment. 4th edition. By GEORGE R. HERRMANN. 652 pp., $12.50. The C. V. Mosby Co., St. Louis, Missouri.

Genetics and Disease. By TAGE KEMP. 330 pp., Kr. (bound) 30.00. Ednar Munksgaard, Ltd. Copenhagen, Denmark.

Gifford's Textbook of Ophthalmology, 5th edition. By FRANCIS HEED ADLER. 488 pp., $7.50. W. B. Saunders Co., Philadelphia, Pa.

Handbook of Tropical Dermatology and Medical Mycology. Vol. 1. Edited by R. D. G. PH. SIMONS. 845 pp., $15.00. Elsevier Press, New York.

A Manual of Clinical Allergy. By JOHN M. SHELDON, ROBERT G. LOVELL AND KENNETH P. MATHEWS. 413 pp., $8.50. W. B. Saunders Co., Philadelphia, Pa.

Praktische Arbeitsphysiologie. By GUNTHER LEHMANN. 352 pp., Ganzleinen DM 33. Georg Thieme Verlag, Stuttgart, Germany.

Säuglgs-und Kleinkindpraxis für Nichtspezialisten. By JULES R. FREYFUS. 74 pp., 6.25 Fr. Verlag Paul Haupt, Bern, Switzerland.

Treatment of Mental Disorder. By LEO ALEXANDER. 507 pp., $10.00. W. B. Saunders Co., Philadelphia, Pa.

Words into Type. Based on Studies by MARJORIE E. SKILLIN, ROBERT M. GAY and other authorities. 585 pp., $5.00. Appleton-Century-Crofts, Inc. New York.